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Analytical Methodology for the Determination of Kepone (Trademark) Residues in Fish, Shellfish, and Hi-Vol Air Filters

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16. ABSTRACT

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The finfish and archival oyster samples were Soxhlet extracted using diethyl ether/petroleum and ether (1:1 v/v) as the extracting solvent. The finfish livers and entrails were macerated in a Duall tissue grinder containing acetonitrile followed by partitioning of the Kepone into benzene. Shellfish samples were analyzed after extracting the sample with acetonitrile and partitioning the Kepone into benzene. The Hi-Vol air samples were extracted with methanol/benzene (1:1 v/v) The coextracting contaminants were removed by micro-Florisil column chromatography and/or acid digestion. The procedural recovery of Kepone from fortified samples averaged 82%.

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Analytical Methodology for the Determination of Kepone® Residues in Fish, Shellfish, and Hi-Vol Air Filters

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Abstract. The recent discovery of the pollution of the environment with Kepone® has resulted in a tremendous interest in the development of residue methodology for the compound. Current multiresidue methods for the determination of the common organochlorinated pesticides do not yield good quantitative analytical results for Kepone. The extracting solvents are usually of insufficient polarity to extract Kepone from the various media. This article describes some of the recently developed methodology for Kepone in air filters, finfish, finfish livers and entrails, shellfish, and archival oyster samples.

The finfish and archival oyster samples were Soxhlet extracted using diethyl ether/petroleum ether (1:1 v/v) as the extracting solvent. The finfish livers and entrails were macerated in a Duall tissue grinder containing acetonitrile followed by partitioning of the Kepone into benzene. Shellfish samples were analyzed after extracting the sample with acetonitrile and partitioning the Kepone into benzene. The Hi-Vol air samples were extracted with methanol/benzene (1:1 v/v). The coextracting contaminants were removed by micro-Florisil® column chromatography and/or acid digestion. The procedural recovery of Kepone from fortified samples averaged 82%.

The compound Kepone®[2] (Common name: Chlordecone, IUPAC name: Decachloro-octahydro-1,3,4-metheno-2*H*-cyclobuta(*cd*)pentalen-2-one) has recently been of much interest to the pesticide residue analyst as a result of the large scale environmental contamination.

In the anhydrous state the compound is a ketone but on contact with any moisture it rapidly hydrates to the very polar geminal diol (Martin and Worthington 1974). As this hydrated form is in environmental samples, most multiresidue extraction methods for organochlorinated pesticides were not adequate for the quantitative determination of Kepone residues in environmental media.

Few articles were found that contained analytical methods for Kepone. Brewerton and Slade (1964) used benzene to remove the Kepone from apple skins. The determination of GC-9160 (the ethyl levulinate derivative of Kepone also known as Kelevan) in cabbage, lettuce, and citrus fruits (Westlake *et al.* 1970) was accomplished by extracting the sample with methylene chloride and

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[2] Due to the familiarity of the trade name Kepone, the authors will use it in this paper.

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oxidation of the GC-9160 to Kepone. Smith and Arant (1967), reporting on the residues of Kepone in cow's milk, did not discuss the extraction of the residue from the milk, but did discuss the cleanup technique of partitioning the extract between oleum and hexane. Similar techniques were used in the method developed by Allied Chemical Co. for the determination of Kepone residues on banana peels (U.S. Food and Drug Administration 1964, Vol II, Section 180.287).

The effort in this laboratory was to find workable methods for the determination of Kepone residues in collected environmental samples. The Hi-Vol air filters were collected by another agency, analyzed for total particulate and sent to us wrapped in aluminum foil. All finfish samples were received in this lab as frozen whole fish, wrapped in aluminum foil. The shell fish samples were collected by another agency, along with the mother liquor, frozen in cans and shipped to this laboratory. Archival oyster samples were collected and prepared by another agency. We received them ground with sodium sulfate (25% oyster meat/75% sodium sulfate), wrapped in aluminum foil and frozen. All samples contained collection data (date, location, sample type, etc.) for proper identification.

The finfish samples were gutted, chopped, and Soxhlet extracted with diethyl ether/petroleum ether (1:1v/v). The finfish livers and entrails were macerated in the presence of acetonitrile and partitioned against benzene to remove the Kepone from the acetonitrile. Shellfish samples were shucked, drained, and blended with acetonitrile and partitioned against benzene. Archival oyster samples were Soxhlet extracted with diethyl ether/petroleum ether. The Hi-Vol air papers were extracted with methanol/benzene (1:1 v/v).

The air samples did not require any further cleanup in preparation for analysis. The finfish meat, liver and entrail samples and the shellfish samples were cleaned up for injection with micro-Florisil chromatography. The archival oyster samples required acid digestion and micro-Florisil chromatography in preparation for analysis. The solvent systems used to elute the micro-Florisil for the different samples types were adjusted to achieve the maximum possible coextractant removal. Though they could possibly be interchangable, we preferred to use the solvent system that optimized the removal of gas chromatographic interferences from the extract of the different sample types.

The final analysis was accomplished by gas-liquid chromatography using electron capture detectors. Confirmation of the residue identity was accomplished by; a) sample injection on several columns (different liquid phases); b) sample injection on Hall Electrolytic Conductivity detector equipped gas chromatograph; and c) sample injection on a gas chromatographic mass spectrometer.

Apparatus, Reagents and Materials

Apparatus

The Tracor gas chromatographs, Models MT 220 and 222, were operated with column oven and inlet temperatures of 200°C and 230 to 250°C, respectively. The columns used were all 1.8 m × 4 mm id glass, packed with 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas Chrom Q (nitrogen flow rate 45 to 60 ml/min), 3% OV-1 on 80/100 mesh Chromsorb W HP (nitrogen flow rate 50 to 60 ml/min), and 4%

SE-30/6% OV-210 on 80/100 mesh Gas Chrom Q (nitrogen flow rate 60 to 70 ml/min). The ^{3}H electron capture detector was operated at 210°C in the DC mode with the voltage set between 85 and 90% of the standing current. The ^{63}Ni electron capture detector was operated at 260°C in the DC mode with the voltage set at 90% of the standing current.

Equipment

Chromatographic columns, Chromaflex, 22-7 and 22-9, Kontes Glass Co.

N-EVAP, Organomation Assoc., Worchester, Mass.

Hobart food chopper, Model 84142, Hobart Manufacturing Co., Troy, Ohio.

Blender, laboratory model, Waring Products Co., Winstead, Conn.

Sorvall Omni-mixer, Type OM, Ivan Sorvall, Inc., Norwalk, Conn.

Centrifuge, Model HN-S, International Equipment Co., Needham Heights, Mass.

Duall tissue grinder, Number K-885450, Kontes Glass Co., Vineland, N.J.

Reagents and Materials

Kepone—Allied Chemical Co., 87.3% purity, (13.7% water of hydration).

Sodium sulfate, granular, anhydrous—Mallinckrodt, Soxhlet-extracted with dichloromethane and oven dried at 130°C before use.

Florisil®, PR Grade, 60 to 100 mesh.

Solvents (benzene, methanol, acetonitrile, and hexane), pesticide quality or equivalent.

Experimental

Sample Preparation and Extraction

Air (Hi-Vol) Samples

The glass fiber filters from Hi-Vol air samplers were cut into squares (*ca.* 60 × 60 mm). One square was placed into a 125-ml glass-stoppered erlenmeyer flask and extracted with 100 ml of methanol/benzene (1:1 v/v) by shaking for approximately 5 min. The sample extract was then filtered through Whatman #1 filter paper in a Buchner funnel to remove the glass fiber and concentrated or diluted as necessary for electron capture gas chromatographic analysis. Further cleanup of the samples was generally not necessary.

Finfish

The liver and entrails were removed prior to homogenation of the fish in the Hobart food chopper. If the fish were to be analyzed later, the homogenate was stored in a glass container at −10°C.

A 25-g subsample was ground in a 500-ml mortar with sufficient anhydrous sodium sulfate (*ca.* 100 g) to dry the sample, in preparation for Soxhlet extraction. The prepared sample was transferred to a preextracted thimble and Soxhlet extracted with 300 ml of diethyl ether/petroleum ether (1:1v/v) for 12 to 16 hr. Upon completion of the extraction the extractor tube was replaced with a Snyder column, and the extract was concentrated to approximatley 50 ml using the same heating mantle used during the extraction. This concentrated extract was quantitatively transferred to a 100-ml volumetric flask using 1% methanol/benzene. A screening injection was used to determine if any further cleanup of the sample would be necessary. Sample cleanup was accomplished by micro-Florisil column chromatography, which is described in the Sample Extract Cleanup section.

Finfish Livers and Entrails

Large samples were homogenized in a Sorvall Omni-mixer at high speed for approximately 2 min. When only a small amount of sample was available, such as with liver, a 500-mg sample of the tissue was macerated in a motor driven Duall tissue grinder with 2.5 ml of acetonitrile as the extracting solvent. After centrifugation to separate the macerated tissue from the solvent the solvent was removed by pipet and placed in a 50-ml centrifuge tube. The maceration/extraction step was then repeated an additional two times, combining the extracts in the same 50-ml centrifuge tube. The resulting extract was mixed with 25 ml of 2% aqueous sodium sulfate and extracted by partitioning against 5 ml of benzene. This extraction was performed twice more with 2 ml portions of benzene. The three benzene extracts were combined in a 10-ml concentrator tube and evaporated to 0.1 ml with a gentle stream of nitrogen. An additional 0.5 ml benzene was used to wash the walls of the tube and concentrated again by the same procedure to approximately 0.1 ml. Sample cleanup was accomplished by micro-Florisil column chromatography.

Whole Clams and Oysters

The clams and oysters were thawed, shucked and drained. The meat was homogenized in a blender at high speed for 2 to 3 min. If the analysis was to be done at a later time, this homogenate was stored at −10°C.

A 10- to 30-g subsample of the homogenate was blended with 50 ml of acetonitrile for 2 min at high speed in a Waring blender. The acetonitrile extract was vacuum filtered through sharkskin paper, without making any attempts to remove the solid particulate material on the sides of the blender. The sides of the blender jar were then scraped with a spatula, allowing the solid material to fall to the bottom of the jar, and a second extraction with an additional 25 ml of acetonitrile, was performed for 1 min. After filtering the second extract through the same filter, the combined extract was transferred into a 1000-ml separatory funnel containing 300 ml of distilled water, 5.0 ml of aqueous saturated sodium sulfate, and 50 ml of benzene. The separatory funnel was shaken for 2 min. The aqueous layer was discarded and the benzene phase was washed twice with 50 ml

of distilled water. The extract was prepared for micro-Florisil chromatography cleanup by passing through a funnel containing *ca.* 5 g of anhydrous sodium sulfate and concentration by nitrogen blowdown.

Archival Oyster Samples (Stored in Sodium Sulfate)

Approximately 30 g of the sample (25% ground tissue, 75% sodium sulfate) was further mixed with 100 g of anhydrous sodium sulfate and then Soxhlet extracted using the same solvent system and method described earlier for finfish. As the levels of Kepone in these samples were low, cleanup of these samples was always necessary. Acid digestion and micro-Florisil column chromatography were used in combination to prepare the samples for injection.

Sample Cleanup

Cleanup of Archival Oysters

A portion of the extract equivalent to 500 mg of the original oyster tissue (not original sample size, as it contained both oyster and sodium sulfate) was evaporated just to dryness under a gently stream of nitrogen in a 15-ml centrifuge tube. The addition of 1 ml concentrated sulfuric acid was followed by shaking the tube for 1 min on a Vortex apparatus. At this point the sample was allowed to set for 10 to 15 min. One ml of benzene, previously saturated with concentrated sulfuric acid, was added and the tube was mixed again for 1 min on a Vortex apparatus. After separation of the phases, the acid layer was removed by means of a disposable pipet and replaced with another 1-ml portion of concentrated sulfuric acid. If, after mixing for another minute, the acid layer did not remain colorless, the procedure was repeated until the acid layer remained colorless. The benzene was then washed with 1 ml of benzene-saturated water. The organic layer was dried by addition of sodium sulfate to the centrifuge tube and careful shaking. The organic solvent was removed and concentrated by nitrogen blowdown to 0.5 ml in preparation for micro-Florisil cleanup.

Preparation and Elution Procedure for micro-Florisil Column Cleanup

A chromaflex column (size 22-7 or 22-9) was loosely plugged with Soxhlet-extracted glass-wool, packed with 1.6 g of Florisil and topped with 1.6 g of anhydrous sodium sulfate. The column was eluted with 25 ml of methanol/benzene (1:1v/v), air dried, and activated in an oven at 130°C for at least 16 hr.

As the columns were needed, they were removed from the oven, cooled to room temperature, and washed with 10 ml of petroleum ether. When the wash solvent (discard) reached the top of the sodium sulfate layer of the column, the sample (0.5 ml of solution, equivalent to 500 mg of the original sample) was placed on the column. The sample tube was rinsed with two 0.5-ml portions of Solvent I (described below). These two rinsings and the rest of Solvent I were

added to the column and collected as Fraction I. This fraction does not elute Kepone. Fractions II and III consisted of the elution with Solvents II and III (as applicable). These fraction(s) elute Kepone from the column. All eluates were collected in 50- or 100-ml centrifuge tubes.

For finfish meat, livers and entrail samples, Solvent I was 25 ml of petroleum ether and Solvent II was 40 ml of methanol/acetonitrile/benzene/hexane (1:2:4:93 v/v). For clam and oyster samples, Solvent I was 25 ml of petroleum ether, Solvent II was 12 ml of benzene followed by 12 ml of methanol/benzene (1:9 v/v), and Solvent III was 24 ml of methanol/benzene (1:99 v/v). For the archival Oyster samples the Solvent I was 7 ml of methanol/benzene/hexene (2:4:94 v/v/v) and Solvent II was 30 ml of methanol/acetonitrile/benzene/hexane (1:2:4:93 v/v/v/v).

Upon completion of the Florisil elution, all samples were adjusted to the proper volume while making certain that the injection solvent was at least 1% methanol in benzene. The samples were concentrated by nitrogen blowdown or steam temperature solvent evaporation using micro-Snyder columns.

Kepone Identity Confirmation

Three schemes of identification were used for Kepone. Confirmation of the identity by retention time (absolute and relative to Aldrin) on differing liquid phase columns by electron capture detector-equipped gas chromatograph was accomplished on all samples. Some of the samples were confirmed by detection on a Hall Electrolytic Conductivity Detector equipped gas chromatograph operated in the chlorine mode, indicating that the sample did have a component that elutes at the correct retention time containing chlorine. Further confirmation of the identity of the residue was accomplished by gas chromatographic mass spectrometry (gc/ms) using a chemical ionization source (CI), operated in the multiple ion detection mode (MID) monitoring for ions of m/e of 489, 490, 491 and 492. These ions are characteristic of Kepone in the CI mode with methane reagent gas.

Results and Discussion

During the course of the experimental work, abnormal solvent effects were noticed on the electron capture detector equipped gas chromatograph. If a low boiling, non-polar injection solvent (hexane or petroleum ether) was used, "ghost peaks" were often noticed in subsequent chromatograms. When benzene was used as the injection solvent, the problem disappeared. Subsequently it was noticed that the sensitivity of the response to Kepone was drastically increased if methanol was added to the benzene prior to injection on the gas chromatograph. Attempts were made to establish if the nature of Kepone was changed upon addition of the methanol. But as the retention time of Kepone on all of the columns used and the gas chromatographic/mass spectrum of the compound did not change upon the addition of methanol, no chemical change in the compound's structure could be proven. (This enhanced sensitivity was also noticed

Table 1. Relative retention data (RRT) on various liquid phases (relative to aldrin)[a]

Liquid phase	RRT_A	Oven temp. (C°)	Carrier flow rate (ml/min)
[b]3% OV-1	2.91	180	70
	2.81	200	70
5% QF-1	2.65	180	45
5% OV-210	2.61	180	60
4% SE30/6% OV-210	2.58	200	70
[b]1.5% OV-17/1.95% OV-210	2.85	200	60

[a] References:
Thompson *et al.* 1975.
U.S. Environmental Protection Agency, Section 4, A, (6) (1974)
[b] Columns used most often

with the toluene/ethyl acetate solvent system used by Moseman *et al.* (1977).) For this reason, the composition of the injection solvent has been stressed.

The relative retention times (RRT) of Kepone on several of the commonly used liquid phases are listed in Table 1. (Thompson *et al.* 1975 and USEPA 1974). Generally Kepone eluted between 8 and 15 min after injection under the conditions listed in the table. Though the RRT of a compound is not by any means a proof of its identity, with the wise selection of two or more differing column liquid phases and the use of a reasonable confirmation scheme the identity of the residue can be quite well established. In this case the two liquid phases selected were of widely differing polarity. (1.5% OV-17/1.95% OV-210 as the primary identification column and 3% OV-1 as the confirmational column). In all cases more than one column liquid phase was used. In some cases three different column phases were used. Over 57% of the samples analyzed by this lab were confirmed by mass spectral techniques and over 22% of the samples were confirmed by Hall Electrolytic Conductivity Detection in the chloride mode.

The analytical results obtained for all of the samples analyzed were further

Table 2. Recovery of kepone

Substrate	Fortification level (ppm)	Number of replicates (N)	Recovery (% and standard deviation)
Whole clam	0.67	2	82%
Whole oyster	0.75	2	80% ± 1.8
Archival oyster	0.047 (0.015 before spike)	2	74%
Finfish livers and entrails	4.0	2	80% ± 3.5
Air	1.00[a]	3	95% ± 4.2
			AVERAGE 82% ± 7.8

[a] μg/sample

Table 3. Elution characteristics of common organochlorine pesticides through the micro-Florisil column

Compound	Amount eluted (ng)	Clam/oyster solvent system	
		Fraction I (%)[a]	Fraction II & III (%)[a]
Arochlor 1254[b]	500	100	0
Aldrin	20	100	0
p,p'-DDE	20	100	0
o,p'-DDT	30	100	0
p,p'-DDT	40	68	32
β-BHC	20	20	80
γ-BHC	10	0	100
Heptachlor epoxide	10	0	100
Dieldrin	20	0	100
p,p'-DDD[b]	20	0	100
Kepone	0	0	100

[a] Percent of the recovered material in the appropriate fraction
[b] Coelutes with Kepone on 4% SE-30/6% OV-210 column

verified by the analytical results obtained independently on the same samples by another laboratory (Moseman *et al.* 1977) using a polytron extractor and 75% ethyl acetate-toluene solvent. These independent quantitative results were used to further verify the extraction efficiencies of the methods described in this paper.

Based on the fortified samples analyzed, these methods are very useful for the analysis of Kepone residues in the various media presented. Increasing the polarity of the extraction solvent a Florisil elution solvent with good specificity and acid digestion can remove the coextracted interferences allowing for the analysis of an otherwise very difficult compound.

Table 2 contains a summary of the procedural recoveries of Kepone from the fortified samples. The fortification levels used were typical of the levels found in the samples analyzed. All of the recoveries were equal to or greater than 80%, with the exception of the recovery from the archival oyster samples, that had been stored for long periods of time in anhydrous sodium sulfate. Recoveries of residues after long term exposure to sodium sulfate is difficult. This particular sample type also had the lowest levels of Kepone of all of the sample types presented.

The sample cleanup was really a difficult problem. Kepone does not quantitatively elute from some of the Florisil columns used in the standard multiresidue methods. For example, the Mills, Onley, Gaither macro-Florisil system (Mills *et al.* 1963) only partially elutes Kepone (<80% recovery). A further problem with this system is that the Kepone elutes in both the 15 and 50% fractions. The recovery of Kepone through the micro-Florisil column system listed for chlorinated pesticides in human or animal tissue and human milk (Thompson 1974, Section 5,A,2) was not quantitative. Minor modification of the solvent system (replacement of hexane by benzene) allowed for good separation with good

gh the micro-Florisil

t system

Fraction II & III (%)[a]
0
0
0
0
32
80
100
100
100
100
100

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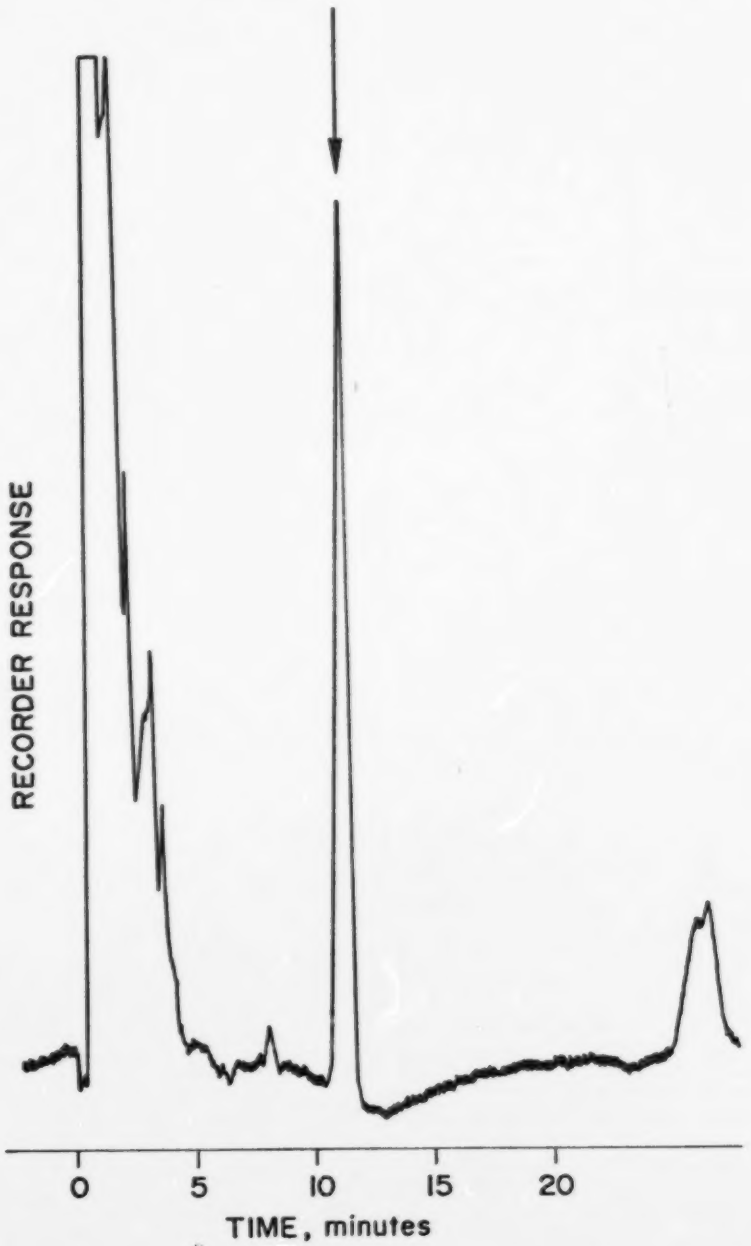


Fig 1. Chromatogram of finfish extract after micro-Florisil cleanup, fractions II and III combined. Injection equivalent to 1.2 mg of finfish tissue. Column: 3% OV-1, nitrogen flow rate: 60 ml/min

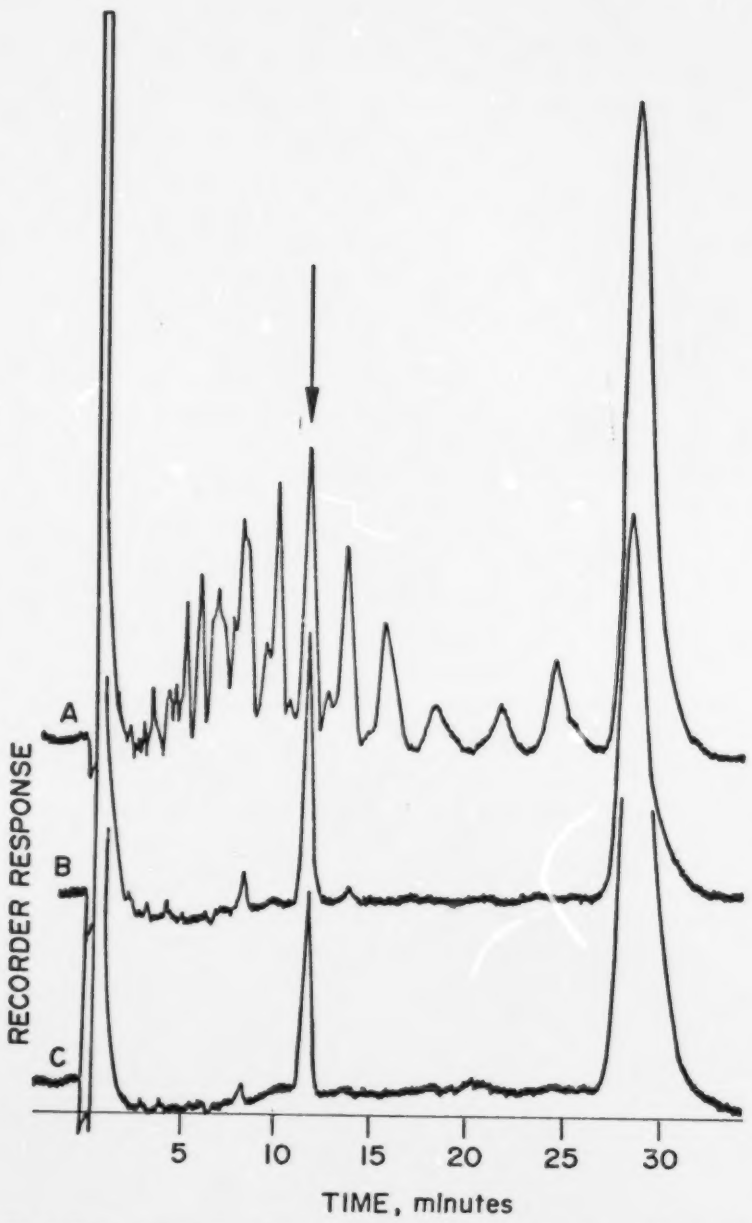


Fig. 2. Chromatograms of shellfish, micro-Florisil fractions: A. Fraction I (no kepone elution in this fraction), B. Fraction II, and C. Fraction III. Injection of 100 μg equivalent of shellfish. Column: 3% OV-1, nitrogen flow rate: 60 ml/min

l no kepone elution in this
nt of shellfish. Column: 3%

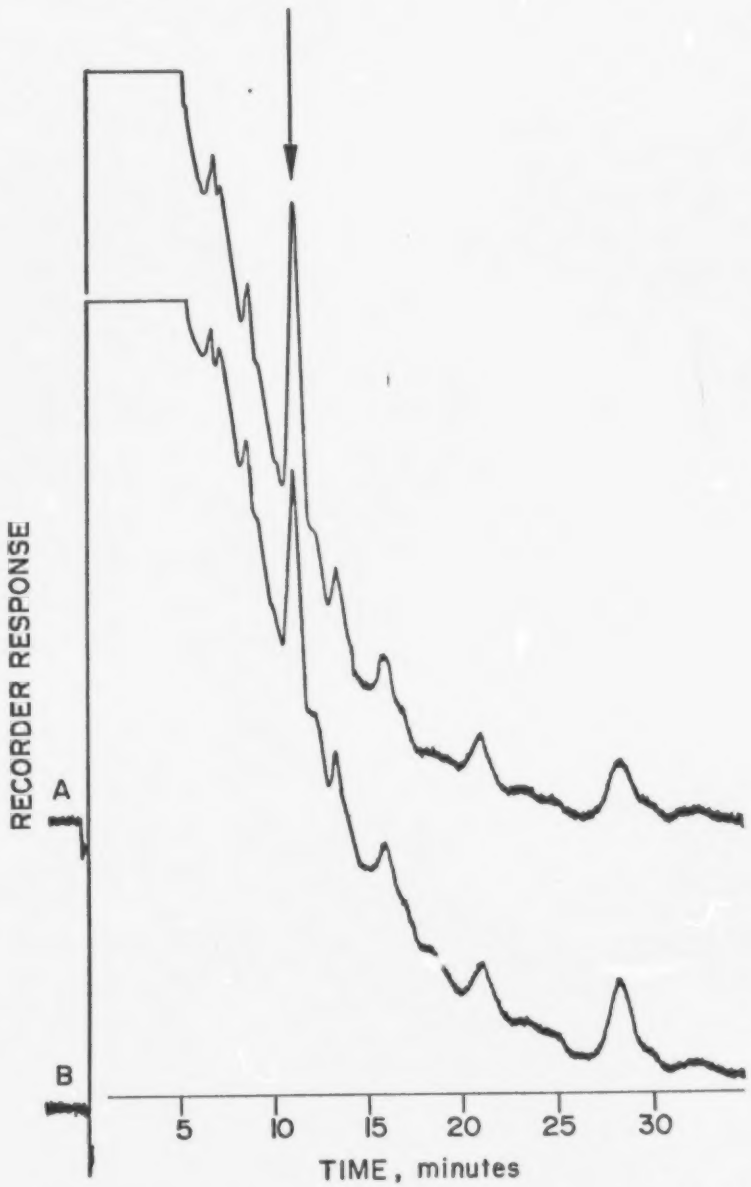


Fig. 3. Chromatograms of archival shellfish extracts, without cleanup. A. With .75 ppm Kepone fortification. B. Control. Injection equivalent to 2.1 mg of shellfish tissue. Column: 3% OV-1, nitrogen flow rate: 60 ml/min

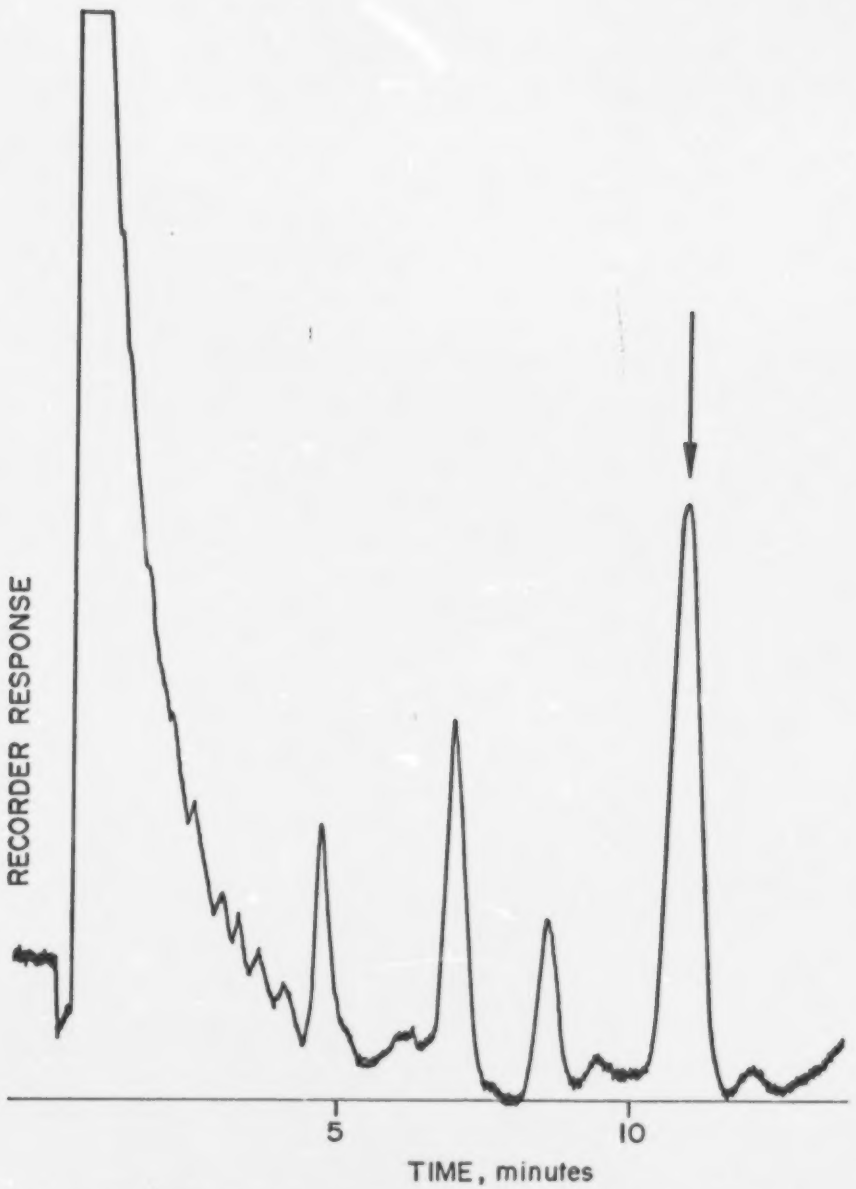
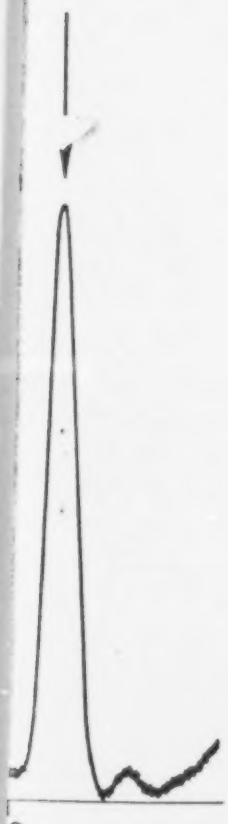


Fig 4. Chromatogram of archival shellfish extract after micro-Florisil and sulfuric acid cleanup. Injection equivalent to 1.1 mg shellfish tissue. Column: 3% OV-1, nitrogen flow rate: 60 ml/min



sulfuric acid cleanup.
flow rate: 60 ml/min

quantitative recovery. Table 3 shows the elution pattern of common organochlorinated pesticides through this system using the solvent system for clams and oysters. In the presentation of the cleanup procedures for the various sample types, a variety of different solvent systems was listed. These variations allowed for the best possible removal of coextractants from the extracts, while permitting acceptable recoveries of Kepone.

In those cases where two Kepone-containing fractions were collected, two possible methods of analysis existed. If the two fractions were analyzed separately the last fraction should have contained less Kepone than the first. If not, it was possible that more Kepone could be eluted from the column with another elution with the first fraction III solvent. Or, the fraction could be combined and analyzed as one fraction, if time was crucial.

Figure 1 shows a typical finfish extract chromatogram after the cleanup of the sample had been performed. In this chromatogram both Fractions II and III were combined prior to injection. As there are no interfering elutants near the retention time of Kepone, quantitative interpretation of the chromatogram is possible.

The chromatograms of the various micro-Florisil fractions from a shellfish sample are illustrated in Figure 2. A sample coextractive having the same retention time as Kepone on the 3% OV-1 column was noted in Fraction 1 (Figure 2,A); however, experiments showed Kepone to elute only in Fractions II and III. Fractions II and III gave clean chromatograms, with an absolute minimum of other eluting compounds (Figure 2,B and C).

The chromatograms in Figure 3 are typical of the chromatograms obtained when any of the animal tissue extracts were injected prior to any cleanup. Figure 3A is a chromatogram of fortified archival oyster sample extract (0.75 ppm). Figure 3B is the chromatogram of the same oyster sample without any fortification. The increased response in the chromatogram of the fortified oyster sample extract marks the retention time of Kepone. As the compound elutes well up on the mound of the solvent/extract response, any interpretation of the crude extract chromatogram would be useless. Figure 4 is the chromatogram of the same unfortified archival oyster sample extract after acid digestion and micro-Florisil column chromatography. In this chromatogram the Kepone residue peak is on the baseline of the chromatogram without any coextracted interference. Certain identification and quantitation are possible with this chromatogram.

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